## Melanomas acquire resistance to B-RAF(V600E) inhibition by RTK or N-RAS upregulation.

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**Public Summary:**
Activating B-RAF(V600E) (also known as BRAF) kinase mutations occur in approximately 7% of human malignancies and approximately 60% of melanomas. Early clinical experience with a novel class I RAF-selective inhibitor, PLX4032, demonstrated an unprecedented 80% anti-tumour response rate among patients with B-RAF(V600E)-positive melanomas, but acquired drug resistance frequently develops after initial responses. Hypotheses for mechanisms of acquired resistance to B-RAF inhibition include secondary mutations in B-RAF(V600E), reactivation of the MAPK pathway, and activation of alternative survival pathways. Here we show that acquired resistance to PLX4032 develops by mutually exclusive PDGFRbeta (also known as PDGFRB) upregulation or N-RAS (also known as NRAS) mutations but not through secondary mutations in B-RAF(V600E). We used PLX4032-resistant cell lines derived from B-RAF(V600E)-positive melanoma cell lines and validated key findings. Knockdown of PDGFRbeta or N-RAS reduced growth of the respective PLX4032-resistant subsets whereas overexpression of PDGFRbeta or N-RAS(Q61K) conferred PLX4032 resistance to PLX4032-sensitive parental cell lines. Importantly, MAPK reactivation predicts MEK inhibitor sensitivity. Thus, melanomas escape B-RAF(V600E) targeting not through secondary B-RAF(V600E) mutations but via receptor tyrosine kinase (RTK)-mediated activation of alternative survival pathway(s) or activated RAS-mediated reactivation of the MAPK pathway, suggesting additional therapeutic strategies.

**Scientific Abstract:**
Activating B-RAF(V600E) (also known as BRAF) kinase mutations occur in approximately 7% of human malignancies and approximately 60% of melanomas. Early clinical experience with a novel class I RAF-selective inhibitor, PLX4032, demonstrated an unprecedented 80% anti-tumour response rate among patients with B-RAF(V600E)-positive melanomas, but acquired drug resistance frequently develops after initial responses. Hypotheses for mechanisms of acquired resistance to B-RAF inhibition include secondary mutations in B-RAF(V600E), MAPK reactivation, and activation of alternative survival pathways. Here we show that acquired resistance to PLX4032 develops by mutually exclusive PDGFRbeta (also known as PDGFRB) upregulation or N-RAS (also known as NRAS) mutations but not through secondary mutations in B-RAF(V600E). We used PLX4032-resistant sub-lines artificially derived from B-RAF(V600E)-positive melanoma cell lines and validated key findings in PLX4032-resistant tumours and tumour-matched, short-term cultures from clinical trial patients. Induction of PDGFRbeta RNA, protein and tyrosine phosphorylation emerged as a dominant feature of acquired PLX4032 resistance in a subset of melanoma sub-lines, patient-derived biopsies and short-term cultures. PDGFRbeta-upregulated tumour cells have low activated RAS levels and, when treated with PLX4032, do not reactivate the MAPK pathway significantly. In another subset, high levels of activated N-RAS resulting from mutations lead to significant MAPK pathway reactivation upon PLX4032 treatment. Knockdown of PDGFRbeta or N-RAS reduced growth of the respective PLX4032-resistant subsets. Overexpression of PDGFRbeta or N-RAS(Q61K) conferred PLX4032 resistance to PLX4032-sensitive parental cell lines. Importantly, MAPK reactivation predicts MEK inhibitor sensitivity. Thus, melanomas escape B-RAF(V600E) targeting not through secondary B-RAF(V600E) mutations but via receptor tyrosine kinase (RTK)-mediated activation of alternative survival pathway(s) or activated RAS-mediated reactivation of the MAPK pathway, suggesting additional therapeutic strategies.